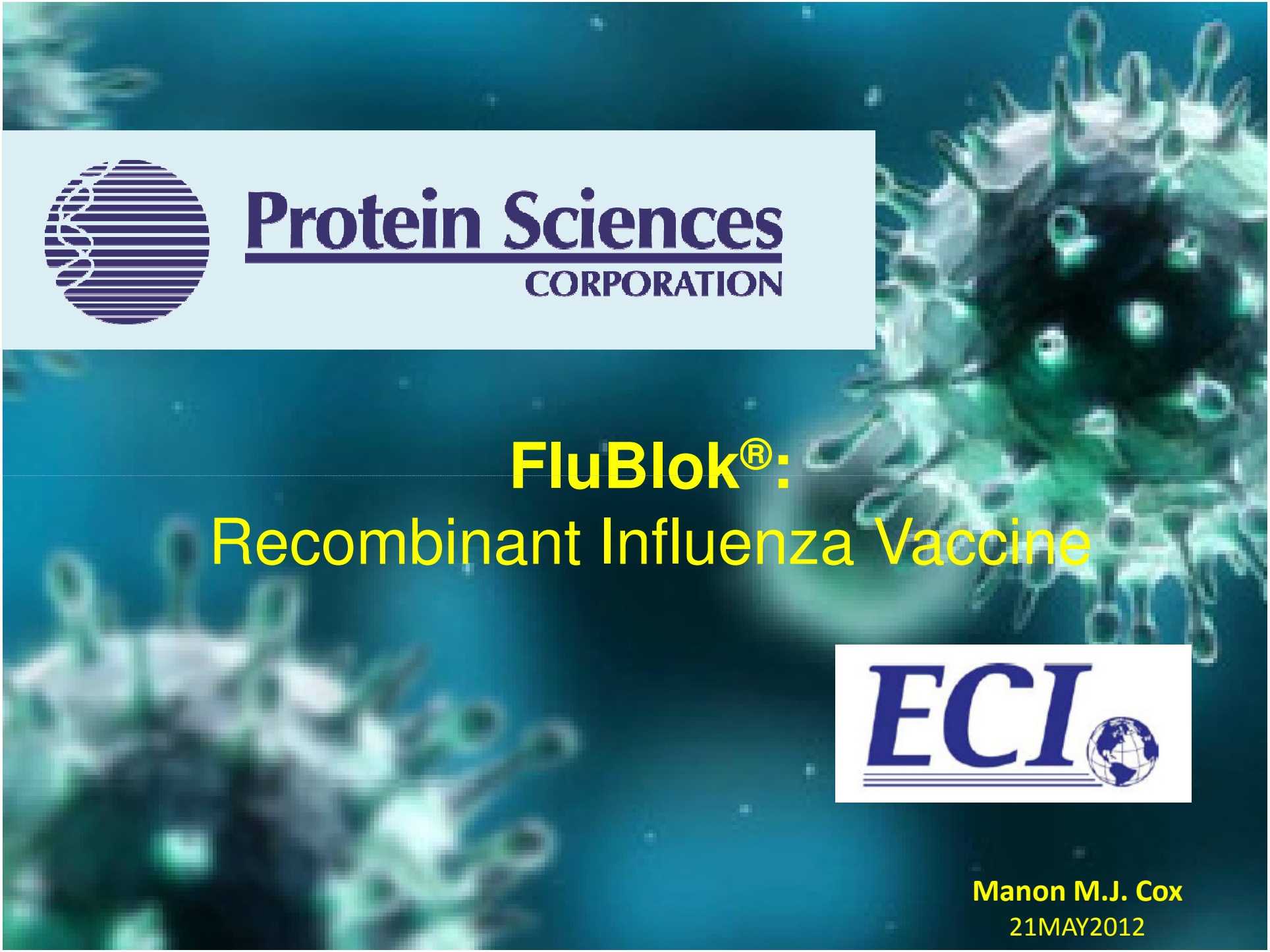
Spring 5-21-2012

Recombinant influenza vaccine

Manon Cox
Protein Sciences Corporation


Protein Sciences
CORPORATION
FluBlok®:
Recombinant Influenza Vaccine
ECI
Manon M.J. Cox
21MAY2012

Vaccine Technology IV
May 20-25, 2012 Albufeira, Portugal

Topics:

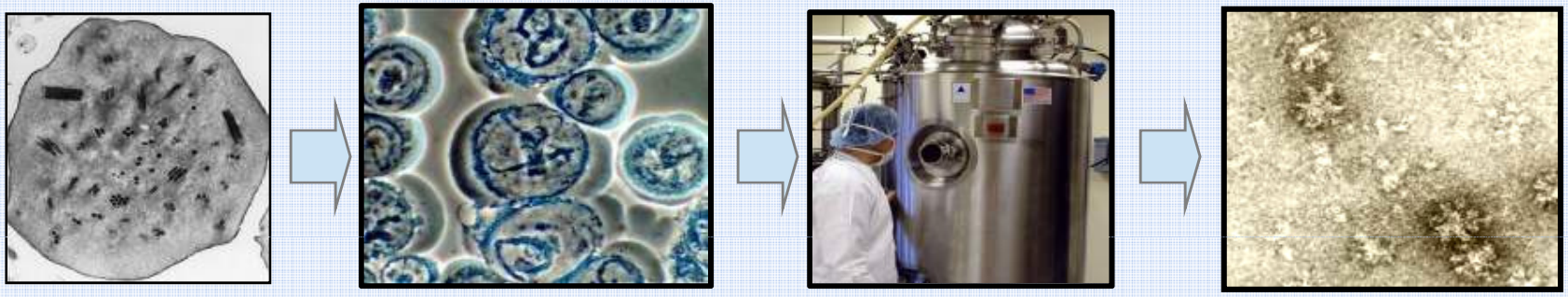
- Baculovirus Technology Platform (BEVS)
- FluBlok®
- Annual Vaccine Adjustments
- Take home lessons

Baculovirus Technology Platform (BEVS)

BEVS Technology

"Enabling products where speed, cost and safety matter"

Baculovirus Expression Vector System (BEVS)



- Engineer baculovirus with the gene of interest (e.g. Hemagglutinin)
- Baculoviruses highly specific to insect cells
- Powerful promoter generates high yield of protein of interest

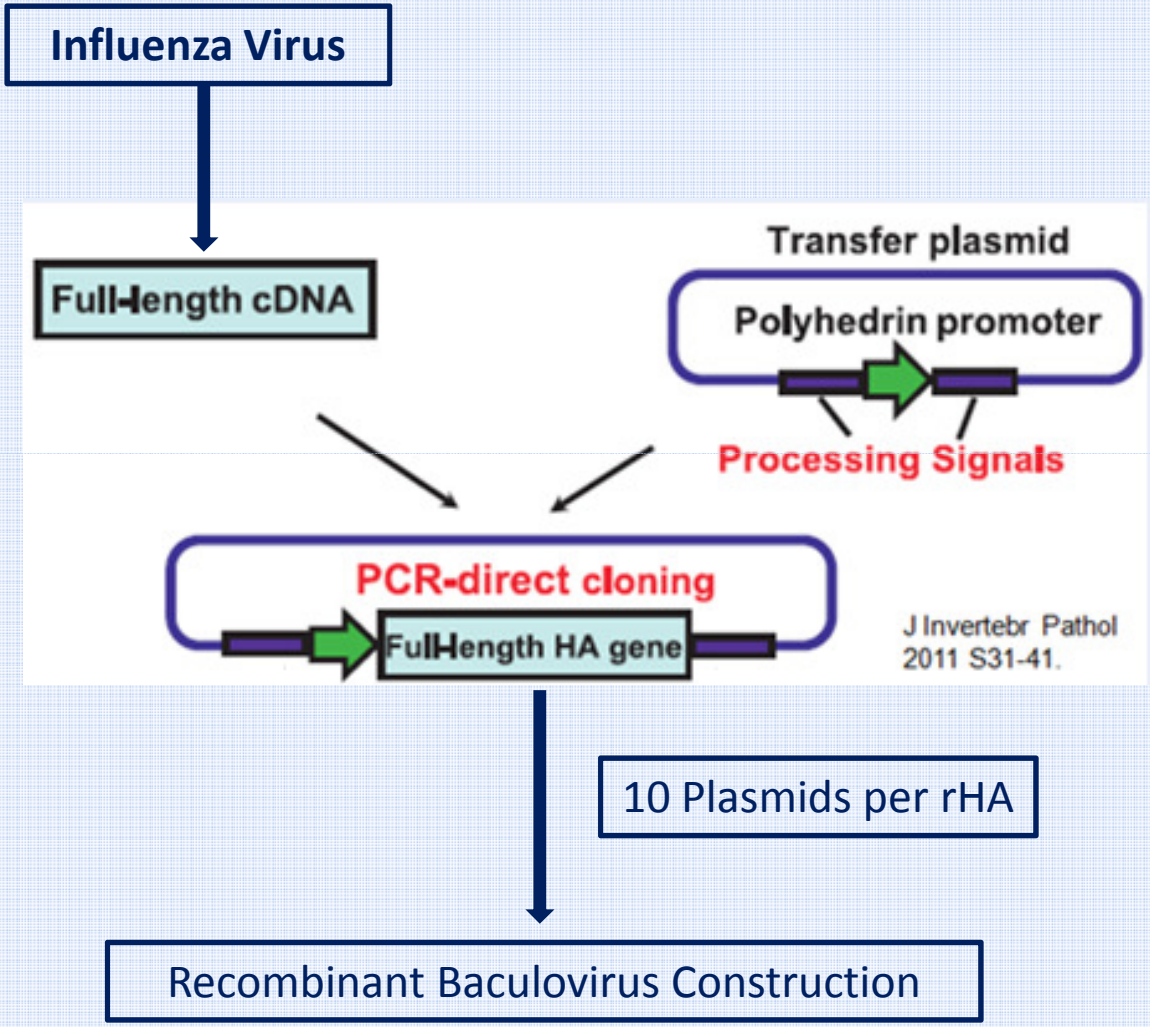
- Culture expression of insect cells in a fermenter
- Infect cells with engineered virus
- Incubate infection for ~48 - 72 hours

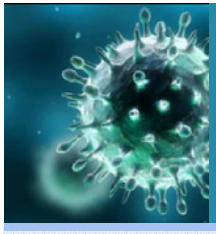
- Protein forms rosettes
- Purify protein to > 90% into final product
- Formulate with PBS into vaccine

FluBlok® Approval → Validation

Virus to Transfer Vector – 3 Days

Influenza Virus
↓
RNA Extraction
↓
RT-PCR of Full Length HA
↓
Ligation into Transfer Plasmid
↓
Transformation into *E. coli*
↓
Colony PCR
↓
Plasmid Preps
↓
Restriction Analysis
↓
Sequencing

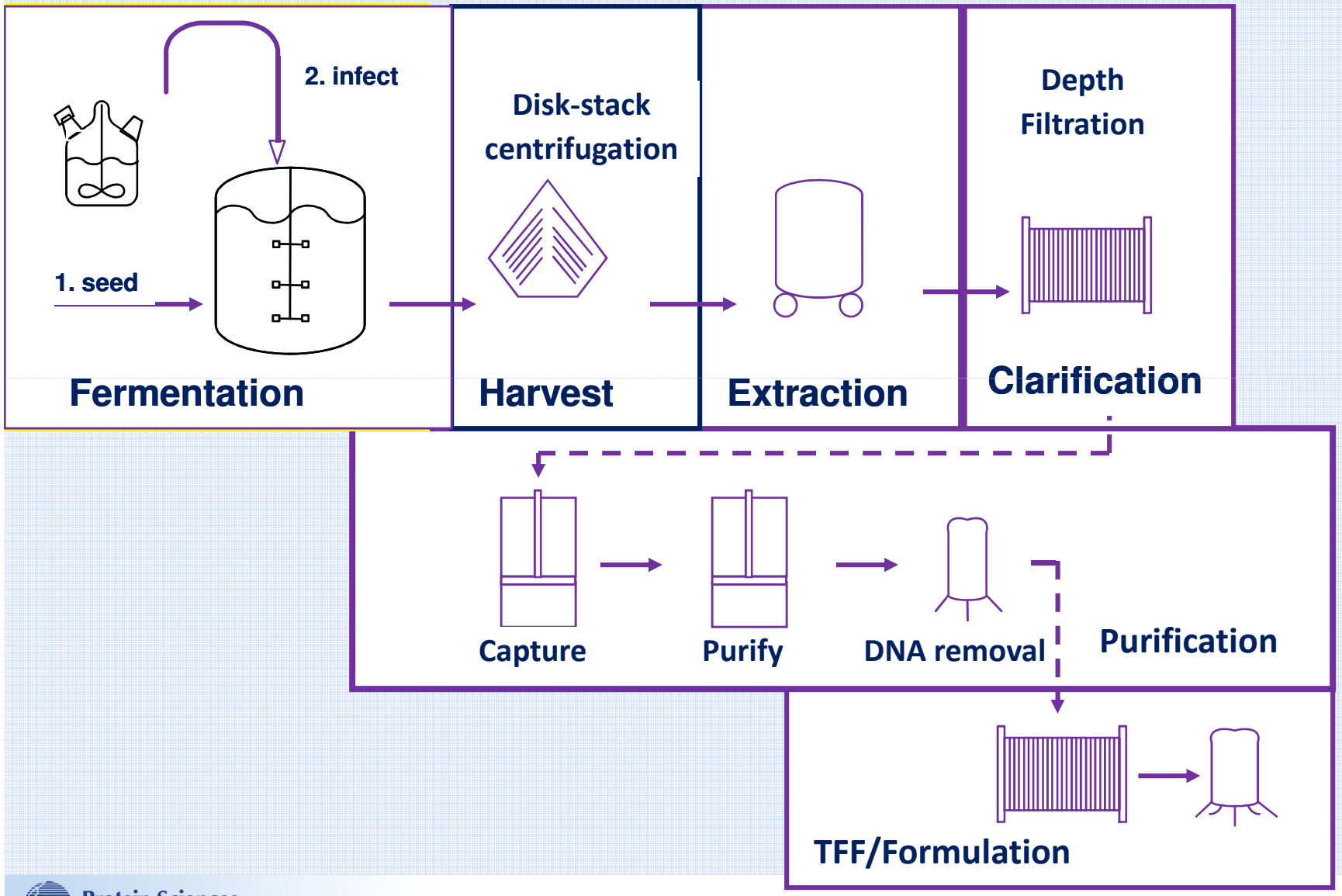


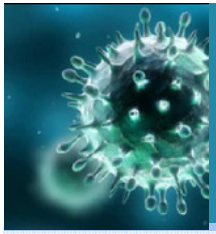


From Influenza Virus to Recombinant Baculovirus Bank

Step	Action	Time
Transfer Vector Preparation	From influenza virus to rHAs gene in transfer plasmid.	3 days
Recombinant baculovirus construction. Clone selection. Virus bank generation.	From transfer plasmid to 300 ml of P3 recombinant baculovirus.	22 days
Virus bank freeze down.	From P3 recombinant baculovirus to frozen vials. **Material from PD to GMP manufacturing**	1 day

Universal Purification Process





Insect Cell-Produced Products & Regulatory Approval Status

Cervarix®

First insect cell product approved by FDA

- Papillomavirus vaccine
- Approved in EU & Australia in 2007
- Approved in U.S. October 2009

Now Approved

Cervarix®

Human Papillomavirus Bivalent (Types 16 and 18) Vaccine, Recombinant

gsk GlaxoSmithKline

Provenge®

Second insect cell product approved

- Prostate cancer immunotherapy
- Approved May 2010

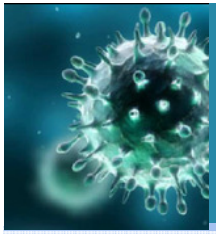
PROVENGE® (sipuleucel-T)

Dendreon

Approvals remove a "barrier" for insect cell-based production platform from regulatory viewpoint

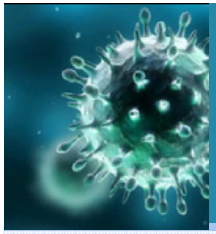
FluBlok
Recombinant Influenza Vaccine

Protein Sciences CORPORATION



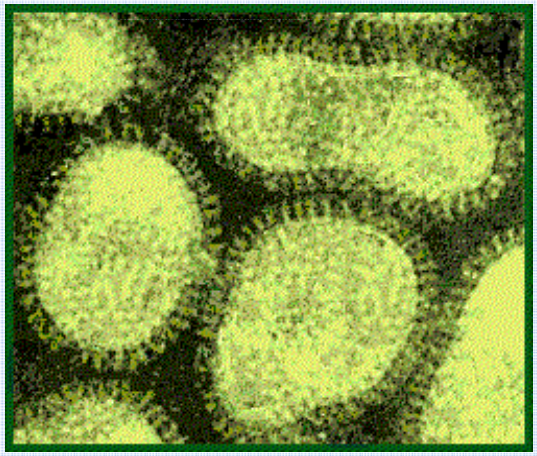
FluBlok

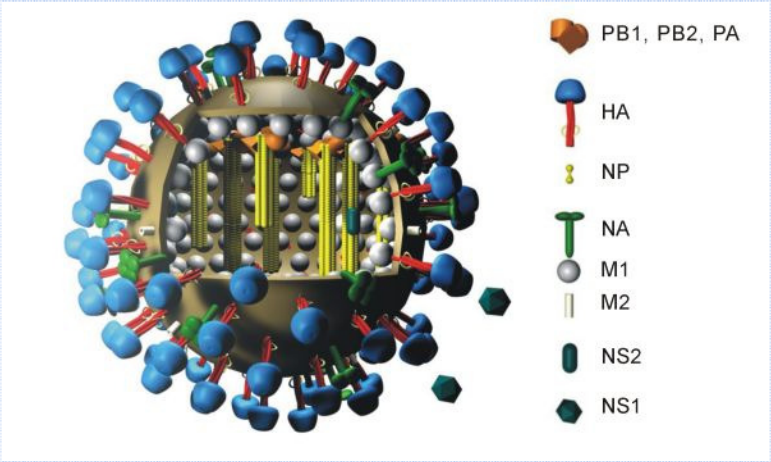
- First recombinant influenza vaccine
- First cell-based influenza vaccine in U.S.
- FDA licensure imminent…
  - *No additional safety or efficacy studies required – FDA letter 01/11/10*
- The pandemic solution
  - Only pandemic vaccine that can be quickly manufactured and/or transferred to and manufactured in other countries



Influenza Vaccine: HA = Major Surface Protein

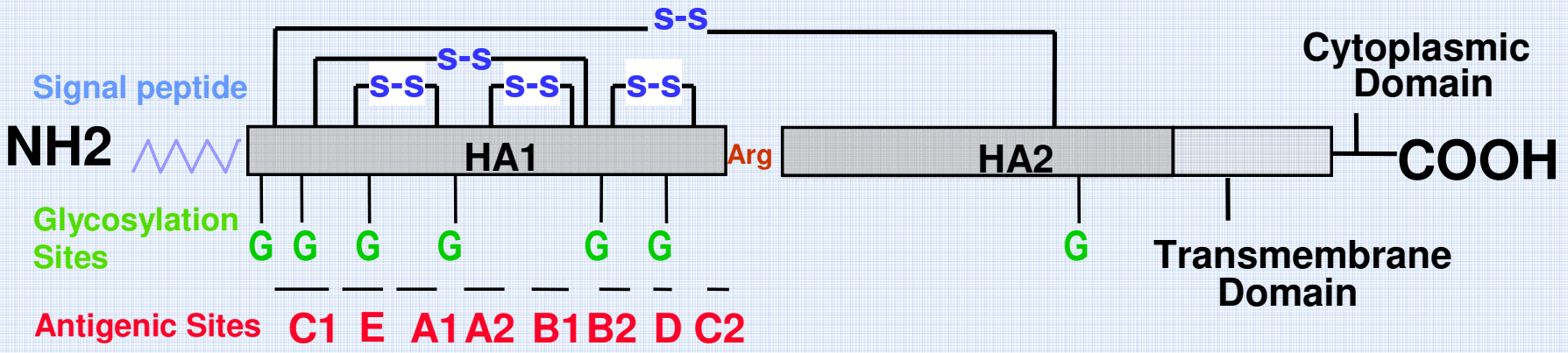
- **HA *(Hemagglutinin)*:**
  - Coat of the influenza virus
  - Antibodies against HA protect against influenza
  - Changes in HA require annual update of vaccine



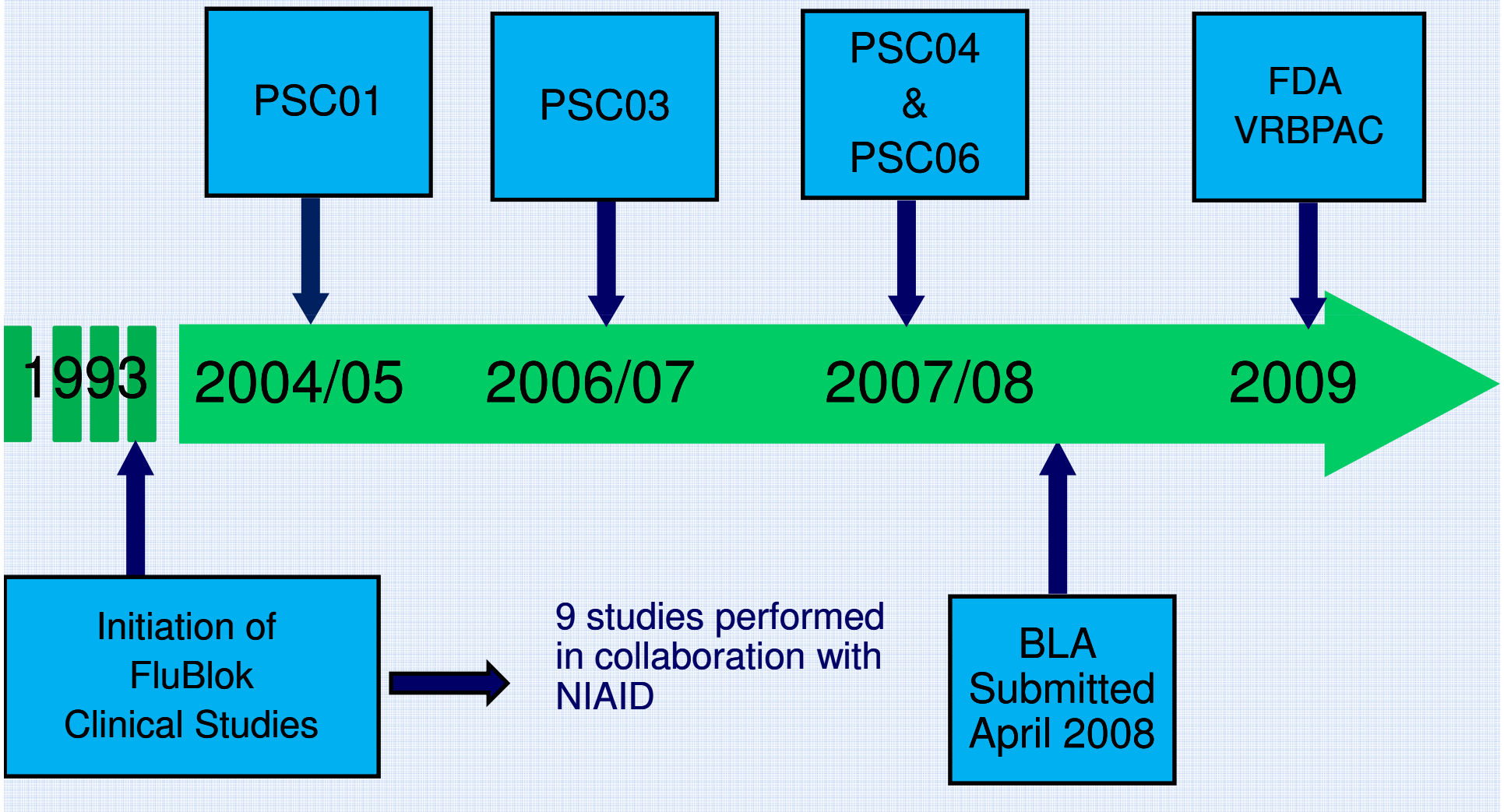


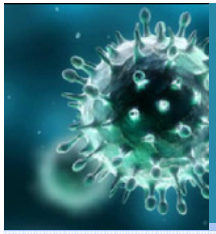
Hemagglutinin properties

- Trimeric integral membrane protein
- Cleavage of HA with host protease into HA1 and HA2 needed for fusion activity
- HA1 and HA2 linked by disulfide bonds
- Contains four antigenic sites (A, B, C, and D)
- Contains many glycosylation sites
- Hydrophobic transmembrane domain



FluBlok Development Timeline

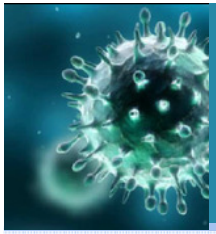




Safety & Immunogenicity of FluBlok Potential Benefits *(3x45µg rHA)*

- Influenza rHA antigens are produced in insect cells – protein based vaccine with low endotoxin content
- rHA protein is highly purified and does not contain egg protein or other contaminants from eggs
- Selection or adaptation of influenza virus strains that produce at high levels in eggs is not required =>the best genetic match
- Cloning, expression and manufacture of FluBlok within 2 months
- FluBlok does not require large amounts of embryonated chicken eggs
- Manufacturing of FluBlok does not require biocontainment facilities
- Manufacture of rHA does not include formalin inactivation or organic extraction procedures

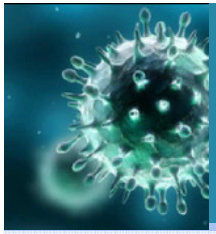
Annual Vaccine Adjustments



Seasonal flu vaccines: constant change.

Flu season (Northern Hemisphere)	WHO recommended vaccine strains		
	H1	H3	B
2010-11	A/California/07	A/Perth/16	B/Brisbane/60
2009-10	A/Brisbane/59	A/Brisbane/10	B/Brisbane/60
2008-09	A/Brisbane/59	A/Brisbane/10	B/Florida/04
2007-08	A/Solomon Islands/03	A/Wisconsin/67	B/Malaysia/2506
2006-07	A/New Caledonia/20	A/Wisconsin/67	B/Malaysia/2506
2005-06	A/New Caledonia/20	A/California/7	B/Jiangsu/10

At least one vaccine component strain changes with every new flu season



Manufacturers must respond to seasonal strain changes quickly

December

New virus available from CDC (Southern Hemisphere 2010 H3 component)

February

New Virus Announced as Northern Hemisphere H3 component

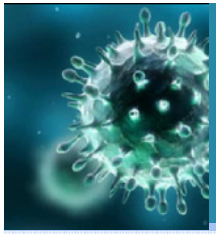
May

SRID assay reagents Available from CBER

August

Flu vaccination season begins

Universal Production Process Needed

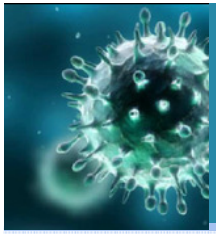


Individual process steps: sensitivity to seasonal changes

upstream

downstream

Step	Isotype variability (H1, H3, B)	Seasonal strain variability	Variable parameters
Fermentation	universal		
Harvest	universal		
Extraction	yes	infrequent	pH; [detergent]
Clarification	yes	no	DF Filter type
IEX	yes	often	pH; [salt]; column type; [detergent]
HIC	yes	some	pH; [salt]; [detergent]
Q membrane	universal		
TFF	universal		



SOP RG0006: Guidance for process development

- Applied to the process development for each new FluBlok component

- Developed with FDA guidance to define acceptable modifications in downstream process parameters within the scope of a validated process.

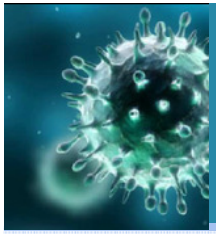
- Provides assurance to FDA that:
  1. any process changes are *necessary and minimal.*
  2. process performance remains consistent season to season

- Provides guidance to process development so that:
  1. development procedures and acceptance criteria are clearly defined.
  2. process changes within R00006 do not require regulatory approval

- Process changes outside of RG0006 require Change Control

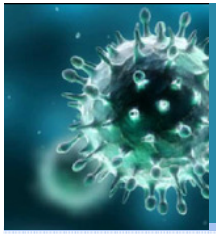
SOP RG0006 guides the work flow and defines the downstream process *design space*

Challenges on Approval Path



CMC Challenges on Path to Approval

- **CMC**
  - Novel Cell Substrate
  - PAI inspection
  - Process Validation



Novel Cell Substrate

Inherent Safety ?

- **Baculovirus**
  - Daily exposure - typical serving of coleslaw contains 112 million polyhedra (each polyhedron contains multiple baculoviruses)[1]
  - Limited Host Range (Lepidopteran Species of Insects)
  - Do NOT Replicate in Mammalian Cells
- **Insect Cells**
  - Virtually No Known Adventitious Agents Can Replicate in both Insect Cells and Mammalian Cells
  - Arboviruses are Rare Exceptions (West Nile Encephalitis)
  - Derived from Non-biting Insects – Low Adverse Events

[1]Heimpel et al (1973) Environmental Entomology, vol2 (1), pp. 72.

How Might Other Products Impact Yours?

Now Approved

Cervarix®

Human Papillomavirus Bivalent (Types 16 and 18) Vaccine, Recombinant

gsk GlaxoSmithKline

Must screen for Nodavirus

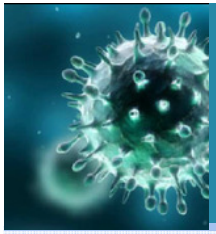
JOURNAL OF VIROLOGY, Oct. 2007, p. 10890–10896 Vol. 81, No. 20
0022-538X/07/$08.00+0 doi:10.1128/JVI.00807-07

Latent Infection of a New Alphanodavirus in an Insect Cell Line▽

Tian-Cheng Li,[1]* Paul D. Scotti,[2] Tatsuo Miyamura,[1] and Naokazu Takeda[1]

Department of Virology II, National Institute of Infectious Diseases, Gakuen 4-7-1, Musashi-Murayama, Tokyo 208-0011, Japan,[1] and Waiatarua, Auckland, New Zealand[2]

Received 14 April 2007/Accepted 26 July 2007



How Might Other Products Impact Yours?

Porcine circovirus DNA in rotavirus vaccine

March 23, 2010

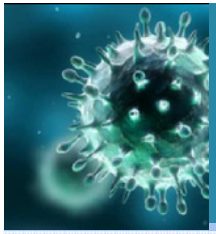
The US Food and Drug Administration has recommended that administration of the Rotarix vaccine, which protects against rotavirus infection, be suspended. This action comes after an independent research group found that the vaccine contains DNA of porcine circovirus type 1.

Initiate screen for all known/unknown viruses

Pre-approval inspections

- **PAI #1 - July 2008**
  - 17 observations (quality systems/manufacturing consistency)
  - Response submitted August 2008
- **PAI #2 – October 2009**
  - 7 observations
  - Response submitted November 2009

Quality Systems remain area of attention



Process Validation

- **Process Validation (PV)**
  - Challenge: Product changes annually (process; product stability etc.)
  - Drug Substance PV now complete

Year	H1	H3	B
2007/08	√	**Failed clinical consistency**	√
2008/09	√	√	√
2009/10	√	√	**+/-**

  - Drug Product PV recently completed

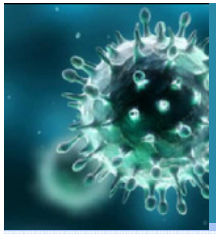
Summary: FluBlok® Regulatory Status

- *FDA is committed to FluBlok approval, however, consistency in product quality and product safety needs to be ensured.*

- FDA recommendation: Start the review clock at that time that all questions are answered.

<u>Open issues</u>:

- Reverse Transcriptase Investigation



Concluding Remarks

- Key advantage = universal “plug and play” process
- Technology may be used for the production of a broad range of protein-based vaccines for both human and veterinary use
- Technology has the potential for reduced manufacturing costs.
  - Existing large scale mammalian cell culture could be deployed for the manufacturing of these vaccines.

Take-Home Lessons

Take-Home Lessons

- Fast Track Designation: A blessing or a curse?
- FDA product approval: “not a slam dunk”!

A recombinant influenza vaccine WILL become a reality thanks to support of contract HHSO100200900106C and perseverance of Protein Sciences employees.

Calvin Coolidge (30th President of the United States): “Nothing in the world can take the place of perseverance…. Persistence and determination alone are omnipotent.”)